

Historic cycles of fragmentation and expansion in the Alpine butterfly *Erebia ligea* (Lepidoptera, Nymphalidae) on the Japanese archipelago, inferred from mitochondrial DNA

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Abstract Phylogenetic relationships among the populations of *Erebia ligea* on the Japanese archipelago were inferred from mitochondrial DNA sequences. The results show a clear division into three lineages on the Sakhalin, Hokkaido and Honshu islands, and also suggest that these lineages have been reproductively isolated from each other since before the last ice age. Further fragmentations were found within these lineages: two genetic lineages on Hokkaido (one found at higher altitudes in the Daisetsuzan Mts. and the other at lower altitudes around some mountain bases) and two on Honshu (one found in the Hida, Yatsugatake and Hakusan Mts. and the other in the Akaishi and Kiso Mts.). Nested clade phylogeographical analysis (NCPA) points to a history of *E. ligea* in the Japanese archipelago which was punctuated by several repeats of allopatric fragmentation and restricted gene flow. The existence of a secondary contact zone shows the same scenario. In the Hida Mts., different haplotypes were detected in the northern and the southern regions, with a secondary contact zone found in the vicinity of the central region. The ancient population was fragmented, reproductively isolated and then differentiated into several populations with distinct haplotypes. Distributions of the two populations then expanded allowing a secondary contact of the two populations, and sporadic distributions were subsequently formed. Repetitive cycles of expansion and reduction in distribution within the Japanese archipelago in response to the glacial cycles of the Quaternary period are suggested.

Key words biogeography, *COI*, *Erebia ligea*, haplotype, *ND5*, nested clade phylogeographical analysis, Quaternary.

Introduction

Alpine organisms are most notably affected by global warming, and therefore reduction of warming by human acts and conservation of these organisms are necessary. In this paper, we focus on alpine butterflies as a representative and assess the history of distribution of populations in the Japanese archipelago during the Quaternary period and their genetic relationships with the continental populations.

1.1 Phylogeographic history of alpine organisms on the Japanese archipelago

There were periods when the Japanese archipelago was connected to the Asian continent during glacial ages and served as a refugium for the animals and plants of continental origin. During glacial ages, many of the arctic and subarctic animals and plants migrated into the Japanese archipelago. During the following interglacial age, ranges of those animals and plants diminished and many of them disappeared. There have been many paleontological studies of fauna based on fossils (Kawamura, 1998), and of flora based on pollen and wooden fossils (Suzuki and Takeuti, 1989).

The major islands that constitute the Japanese archipelago are Sakhalin, Hokkaido, Honshu, Shikoku, and Kyushu

(Fig. 1). The Tartar Strait and Soya Strait are shallow, and they were formed less than 11,000 years ago (Ohshima, 2000). On the other hand, the Tsugaru Strait and Tsushima Strait are deep. Honshu separated from the Korean peninsula and Hokkaido, and became an island during the interglacial age after the Riss glaciation about 150,000 years ago. It is believed that Honshu has not been connected to the continent since then (Ohshima, 2000). Sakhalin and Hokkaido had been connected to the continent almost continuously during a period of 65,000 years from about 75,000 years ago to about 10,000 years ago. They had been parts of the continent for longer periods of time than the periods when they were isolated islands. Thus, during the glacial period geographic conditions allowed organisms to migrate southward from the continent through Sakhalin to Hokkaido (Ono, 1990). On the other hand, alpine organisms that now have an isolated distribution in the alpine region of Honshu had expanded their distribution prior to about 150,000 years ago from the continent, and they are the species that were affected by climate changes since then during the interglacial period and the last glacial period.

High altitude areas of central Honshu and Hokkaido in the Japanese archipelago have had at least two periods when glaciers developed (Iwata, 2003). Reconstruction studies of ancient flora by pollen analysis (for example,

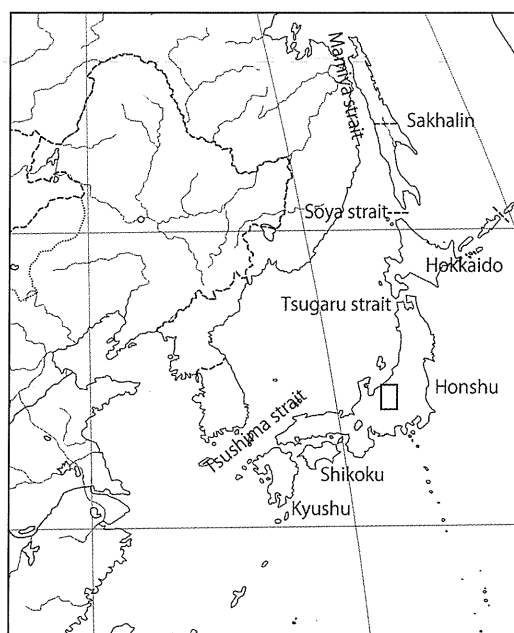


Fig. 1. The Japanese Archipelago.
Major islands and straits are indicated. The small rectangle shows the location of Hida Mts.

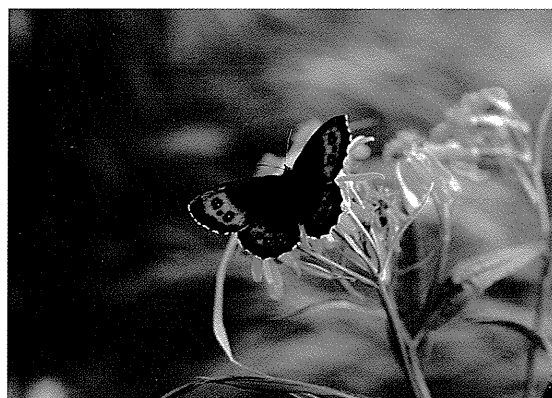


Fig. 2. *Erebia ligea takanonis* (photo courtesy of M. Fukumoto)

Hoshino, 1998) suggest that low altitude areas on Hokkaido were the southern limit of taiga during the last glacial period, and that there were forests richer with *Larix gmelinii* than those in the present northern Sakhalin. In the eastern and northern parts of Hokkaido, the distribution of taiga with *Larix gmelinii* as its main plant were divided in such a way that coniferous forests, grasslands, wet vegetation and alpine tundra formed segregated niches according to altitude and/or geographical features. Thus the areas functioned as refugia for many organisms in the Far East (Ono, 1990).

The limitations on regional biogeographic approaches or studies based on the degree of specialization of external characters for understanding immigration routes of alpine butterflies into the Japanese archipelago, and varying patterns of their distribution on the islands, have been pointed out (Hiura, 1977). Recent studies based on a new viewpoint provided by a molecular phylogenetic approach, circumvent these limitations. Some examples are as follows: studies on the alpine butterflies *Erebia neriene* ssp. (formerly treated as *Erebia nipponica*) (Nakatani *et al.*, 2007a), *Oeneis norna* ssp. (Usami *et al.*, 2011), and also on some alpine plants (Fujii and Senni, 2006).

The process of expansion and contraction of the ranges of organisms due to climate changes in the Quaternary is well known as a process of north-south migration on the Japanese archipelago. According to our studies on alpine butterflies, small populations of both *Erebia neriene* ssp. and *Oeneis norna* ssp. survived during the interglacial period within more than one refugium on the Japanese archipelago. As a result of isolation in each refugium during the warm periods, genetically differentiated populations have survived till now, forming multiple lineages by expanding and contracting their distribution areas as they adapted to later glacial cycles (the multiple refugia model). On the other hand, some species of alpine plants have two lineages in Honshu, northern and southern, and the boundary is in the northeast of the island. As the primary cause of such a distribution character, the following scenario has been suggested. The original population remained in the mountainous areas of central Honshu as a refugium during the interglacial period and later became the southern lineage. A new lineage then migrated into the Japanese archipelago during the next glacial period and formed the northern lineage (the single refugium model).

1.2 Systematics and ecology of the subject species

Erebia ligea (Fig. 2) is widely distributed in the mid to high latitude regions of the Eurasian continent, from the European Alps and Scandinavia in the west through the Ural mountains to the Kamchatka Peninsula in the east. Populations are found in the Japanese archipelago on Sakhalin, Hokkaido and Honshu islands, and are recognized as separate subspecies (ssp. *rishirizana* from Sakhalin and Hokkaido, ssp. *takanonis* from Honshu). In Honshu, this species inhabits grasslands in coniferous forests where species such as *Poa* and/or *Carex* grow, or grasslands just above the timberline, and the distribution ranges are found from 1,700–2,500 m alt. Although *E. ligea* frequently coexists with *E. neriene* in Honshu, *E. ligea* has more limited distribution areas. The upper elevation limit particularly is lower, while the lower elevation limit is

higher, leaving only a narrower zone of suitable elevation. In Hokkaido, *E. ligea* is found at a higher elevation in the Daisetsuzan Mts., and also at the foot of some mountains approximately 1,000 m in height, often along woodland paths. An isolated population is also found at the summit of Rishiri Island, northern Hokkaido. This kind of distribution pattern, present, though sporadically, in wide areas is desirable for a study of phylogeographic history (DeChaine and Martin, 2004; Nakatani *et al.*, 2007a).

2. Materials and methods

2.1 Specimens and collection of molecular information

The authors collected 84 individuals from the Japanese archipelago, and also 12 individuals in Europe, Mongolia, and Russia for reference. The bodies of the sample specimens without wings were preserved in 99% ethanol and kept at 4°C until DNA was extracted.

For genetic markers, we used the NADH dehydrogenase subunit 5 (*ND5*) and cytochrome oxidase subunit 1 (*COI*) genes in mitochondrial DNA. DNA was extracted from the thoracic flight muscles or legs by DNeasy Tissue Extraction Kit (Qiagen, Hilden, Germany). PCR was conducted in a 10 µl reaction system by adding DNA (approximately 40 ng) with reaction constitution as recommended by Applied Biosystems. Thermal cycling profile was one step at 95°C for 10 min; 25 cycles at 95°C for 30 sec, 45°C for 30 sec and 72°C for 1 min 30 sec; one extension step at 72°C for 8 min 30 sec. After amplification, the PCR products were purified with ExoSap-IT (USB, Cleveland, OH, USA). The nucleotide sequences of the amplified DNA fragments were determined by the direct sequencing method with BigDye Terminator v1.1 Cycle Sequencing Kit (ABI, Weiterstadt, Germany) on an ABI Genetic Analyzer. As primers for PCR and direct sequencing, we used V1 (5'-CCT GTT TCT GCT TTA GTT CA-3'), C2 (5'-ATC YTT WGA ATA AAA YCC AGC-3') (Yagi *et al.*, 1999) for *ND5*, and mtD6 (C1-J-1718; 5'-GGA GGA TTT GGA AAT TGA TTA GTT CC-3'), Nancy (C1-N-2191; 5'-CCC GGT AAA ATT AAA ATA TAA ACT TC-3') (Simon *et al.*, 1994) for *COI*. We conducted fragment comparison of 432 bp for *ND5*, 465 bp for *COI*, 897 bp in total.

2.2 Sequence alignments and character statistics

Mitochondrial *ND5* and *COI* sequences were edited and aligned using SeqScape V. 2.11 (ABI, Weiterstadt, Germany). Base frequency homogeneity was tested separately for each dataset with the χ^2 -test using PAUP* 4.0b10 (Swofford, 2002). The χ^2 -test did not reject the hypothesis of homogeneity of nucleotide frequencies in every pair of taxa ($P = 1.00$). To test conflicts in phylogenetic

signal among each dataset we conducted an incongruence length differences (ILD) test (Farris *et al.*, 1994) in PAUP* 4.0b10 with heuristic searches with tree bisection and reconnection (TBR) and 100 random addition replicates for each. The ILD test revealed no conflict between *ND5* and *COI* ($P = 0.58$).

2.3 Nested clade phylogeographical analysis

Nested clade phylogeographical analysis (NCPA) (Templeton *et al.*, 1995; Templeton, 1998, 2004, 2008) can infer past events that affected the population constituents of a species such as past fragmentation, restricted gene flow and range expansion by combining haplotype phylogeny and geographical information on its distribution. We inferred topology among haplotypes by using statistical parsimony methods (Templeton *et al.*, 1992) integrated into the TCS v. 1.21 (Clement *et al.*, 2000). The network is connected with 95% reliability that multiple transfers do not occur at any locus. The relationships between the inferred phylogeny and geographic position can be evaluated by comparison of two distances of the within clade distance (D_c) and the nested clade distance (D_n), and by the interior-tip contrasts (I-T). The significance of the distances defined by NCPA is evaluated using $\alpha = 0.05$. These evaluations were calculated by the GeoDis program (Posada *et al.*, 2000). When the evaluation is significantly large (L) or significantly small (S), they indicate that haplotype/clade distribution is significantly widespread or significantly restricted. Significant I-T contrast for both D_c and D_n values denotes a displaced (L) or restricted (S) distribution of lower versus higher haplotypes/clades.

3. Results

3.1 Genetic difference among the continent, Sakhalin, Hokkaido, and Honshu populations

Distribution frequency of haplotypes and sample sites are shown in Table 1. As a result, 17 haplotypes from the Japanese archipelago (including Sakhalin) and 7 from the continent were detected. Topology of the relationships among haplotypes was inferred using a statistical parsimony method (Fig. 3). Due to the large number of mutational steps separating the continental and the Japanese archipelago haplotypes, they could not be joined in the network with greater than 95% confidence. *COI* (465 bp) fragment comparison indicated that there is about 2.9% (uncorrected pair wise distance) differences of substitutions between major haplotypes of the continental and the Japanese archipelago populations. Also, between the populations in Honshu and Hokkaido, or those in Hokkaido and Sakhalin, there is about 0.8% and 1.6% difference respectively. Within the continent, the difference between

Table 1. Distribution of the haplotypes (*ND5* plus *COI*) of *Erebia ligea* from each sampling range.

Range	Site name	Latitude Longitude	Haplotype														YA000	KI000	AK000
			SA000	DA000	DA010	HL000	HL010	HI000	HI001	HI002	HI003	HI004	HI005	HI100	HI110	HI101			
Sa	Sakhalin	50°34' 142°34'	1																
Da	Daisetsu Mts	43°37' 142°54'		3	2														
HI	Hokkaido (low land)	43°30' 143°16'				4	1												
Hi-N	N Hida Mts	36°46' 137°45'						16	1	1	1	1		1					
Ta	Tateyama Mts	36°32' 137°35'											1						
Hi-S	S Hida Mts	36°31' 137°42'						9						18	4	1			
Ha	Hakusan Mts	36°08' 136°46'						6											
Ya	Yatsugatake Mts	35°59' 138°21'															2		
Ki	Kiso Mts	35°43' 137°49'																6	
Ak	Akaishi Mts	35°29' 138°10'																	6
Total n			1	3	2	4	1	31	1	1	1	1	1	19	4	1	2	6	6
Accession Number (<i>ND5</i>)			AB739587	AB739588	AB739589	AB739590	AB739591	AB739592	AB739593	AB739594	AB739595	AB739596	AB739597	AB739598	AB739599	AB739600	AB739601	AB739602	AB739603
Accession Number (<i>COI</i>)			AB739604	AB739605	AB739606	AB739607	AB739608	AB739609	AB739610	AB739611	AB739612	AB739613	AB739614	AB739615	AB739616	AB739617	AB739618	AB739619	AB739620

populations taken in Europe and those in Mongolia is only 0.2%.

3.2 Nested clade phylogeographical analysis (NCPA) on Honshu populations

It is clear that the populations of Sakhalin, Hokkaido, and Honshu were separated from each other by past allopatric fragmentation. So we studied the distributional transition in Honshu. All haplotypes from Honshu were grouped into a single 3rd level clade (Fig. 5). NCPA showed a significant association between genetic structure and geographic distribution (Fig. 6). There are 5 clades where a significant tendency ($P < 0.05$) of genealogy and geographic location was suggested by NCPA (Table 2).

In the application of NCPA, the age of a clade is the maximum of the ages of the smaller clades nested within it (Templeton *et al.*, 1995). Hence, the older clades are in

general the larger clades with more nested series of clades. Three clades, 1-1, 2-1, and 3-1, were within a nested series of clades, and also appeared to be statistically significant.

(1) Early fragmentation

After migrating into the Japanese archipelago, and spreading out into areas that include the present distribution ranges, they dispersed into three disjunct refugia during warm interglacials, and divided into three lineages, the Sakhalin, Hokkaido, and Honshu lineages (allopatric fragmentation).

(2) The second fragmentation

A panmictic population at an early glacial period divided into three separate populations of the northern-1 lineage (southern Hida Mts. and Yatsugatake Mts.; clade 2-1), northern-2 lineage (northern Hida Mts. and Hakusan Mts.; clade 2-2), and the southern lineage (Akaishi Mts. and Kiso Mts.; clade 2-3) as a result of isolation in three refugia

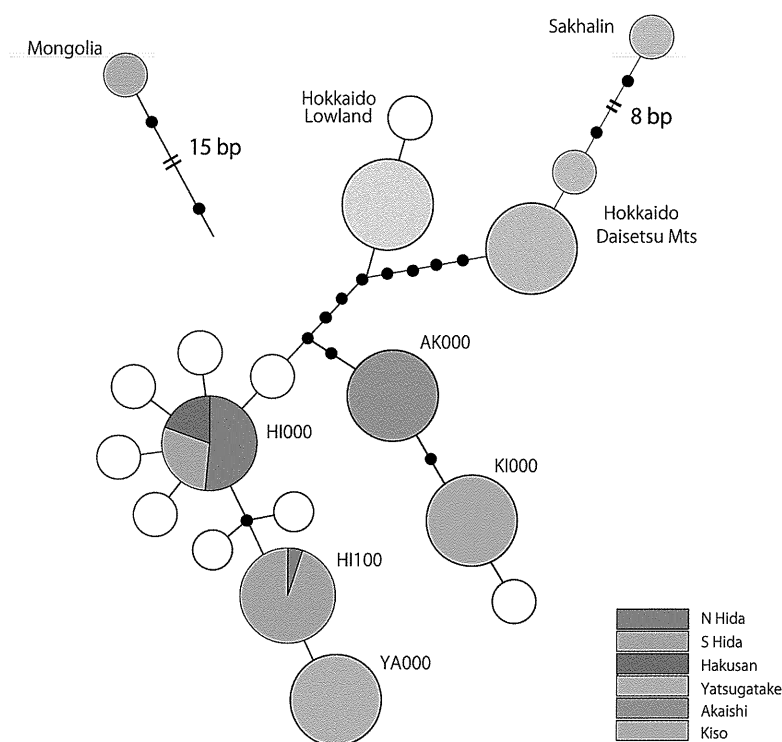


Fig. 3. Statistical parsimony network.

Circles denote haplotypes, the size of the circle reflects the wideness of distribution ranges in 2 levels, small solid circles denote missing or theoretical haplotypes, and a node represents a single mutational step. The pie chart shows the proportions of regions with the same haplotype. Uncolored haplotype was detected in one region only.

during the following warm interglacial period (past fragmentation, clade 3-1).

(3) Isolation by distance

The northern-1 lineage (clade 2-1), which is a 2nd level clade belonging to clade 3-1, is inferred to have two separate populations, the northern Hida Mts. population (clade 1-2), and southern Hida Mts. and Yatsugatake Mts. population (clade 1-1), by restricted gene flow, particularly gene flow restricted by isolation-by-distance.

(4) Fragmentation and habitat islanding

The southern Hida Mts. and Yatsugatake Mts. population (clade 1-1) is fragmented into the Hida Mts. and Yatsugatake Mts. populations.

4. Discussion

4.1 Distributional transitions in the Japanese Archipelago

Based on the inference key of NCPA (Dr Posada's web site, *var.* Jan 06, 2011), the history of *E. ligea* in the

Japanese archipelago was punctuated by several cycles of allopatric fragmentation and restricted gene flow. Inferences of historical processes made by NCPA should be viewed with caution, because the inference keys make qualitative assessments (Knowles and Maddison, 2002; Panchal and Beaumont, 2007; Templeton, 2008). But the presence of traces of secondary contacts of two populations with different haplotypes, found in the area close to Mt. Harinokidake (hereinafter referred to as "Harinoki Gap", Fig. 4), northern Hida Mts., is direct evidence that the *Erebia ligea* populations underwent repeated fragmentation and dispersal as a result of glacial cycles. It suggests that distribution ranges fragmented into separate areas like islands in the past warm period, in each of which a population with characteristic haplotypes evolved, and that later in the glacial period these populations expanded and came into secondary contact with each other, following which, in the next warm period, the distribution ranges have again become like islands. These traces of secondary contact of different haplotypes strongly support the notion that cycles of fragmentation and dispersal of distribution ranges in the Pleistocene took place in the Japanese

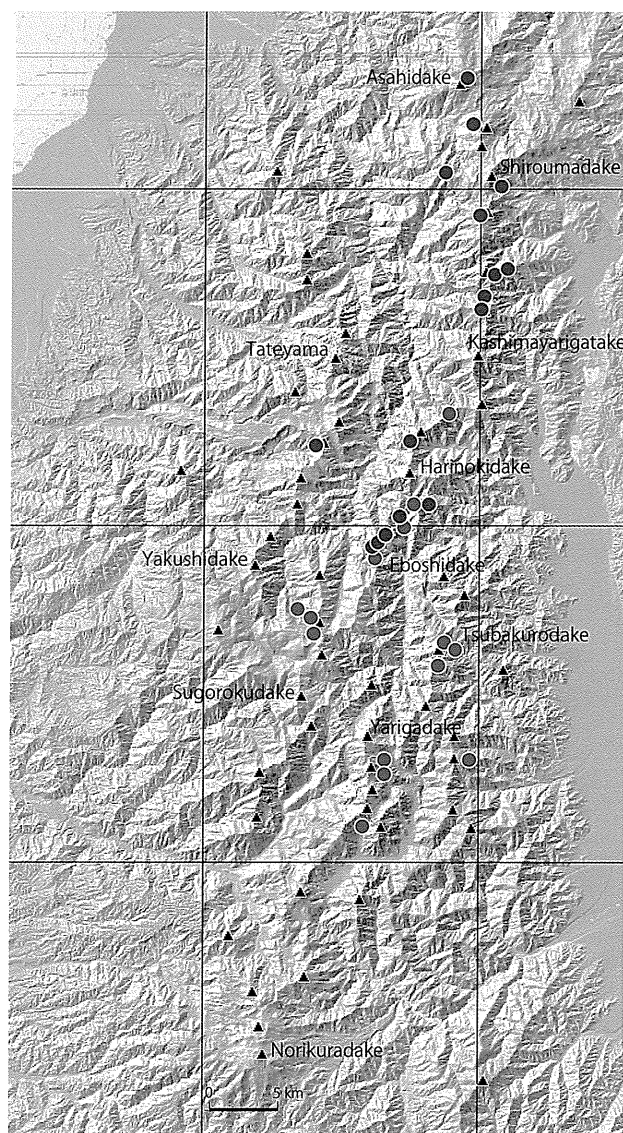


Fig. 4. Sampling sites and geographic distribution of haplotypes from Hida Mts. Red: HI000, blue: HI100.

archipelago. The history is similar to that of *E. neriene* (Nakatani *et al.*, 2007a). The existence of such traces of secondary contacts of different haplotypes observed for two distinct species in Japan strongly supports the notion that cycles of fragmentation and dispersal of distribution ranges in the Pleistocene took place in the Japanese archipelago.

4.2 Population genetic structures as compared with those of *Erebia neriene*

Conceptual distribution maps of major haplotypes and their phylogenetic relationships are shown for *E. ligea* (Fig. 7A) and for *E. neriene* (Fig. 7B). In both species,

clear genetic differentiation can be identified between the populations in the Hida Mts. and Akaishi Mts. The ancestral population, which initially had a single genetic structure, was separated into two populations, one distributed in the Hida Mts. and another in the Akaishi Mts. through the process of a reduction in distribution areas during the subsequent warmer period.

The genetic distances between the two lineages, originating from a refugium in the Hida or Akaishi Mts., are differentiated between the species *E. ligea* and *neriene*, suggesting a possibility that the two species fragmented around different warmer periods. In both species, the Akaishi lineage is closest to the northernmost population in the Hida lineage, allowing for the conclusion that the expansion routes might be similar.

For the populations in the Hida Mts., the major difference between the two species is that *E. neriene* has a unique haplotype, while *E. ligea* is divided into two sub-lineages, north and south, separated with a range of sympatry in the Harinoki Gap. In the Akaishi Mts. conversely, *E. ligea* has a unique haplotype, and *E. neriene* is divided into two sub-lineages with a range of sympatry. Despite the Haku-san Mts. being geographically far distant from the Hida Mts., both species share the same haplotype in the two mountain systems (for *E. ligea*, the northern sub-lineage in the Hida Mts.), suggesting that the populations in the two mountain systems were, up until recently, conducting genetic exchanges in both species.

E. neriene in the Yatsugatake Mts. shares the same haplotype as that in the Hida Mts., while the haplotype of *E. ligea* in the Yatsugatake Mts. differs from that in the southern sub-lineage in the Hida Mts. by only a single base substitution, suggesting a close relationship between the populations of the two regions. In contrast *E. neriene* in the Kiso Mts. shares the same haplotype as that of the Hida Mts., while *E. ligea* in the Kiso Mts. is closely related to the Akaishi lineage. This suggests that different scenarios of distribution transitions should be inferred for the populations of the two species in the Kiso Mts. Concerning the Sakhalin and Hokkaido populations, *E. neriene* populations on the two islands share the same haplotype, indicating recent genetic exchanges. For *E. ligea*, however, there is a large genetic difference between the populations on the two islands, suggesting reproductive isolation ever since the era before the last ice age. In contrast to the widespread distribution of *E. neriene* in the lowland of Hokkaido (particularly at the northern end, where this species is present even on terraces near the seashores), the distribution of *E. ligea* is limited to higher altitudes over 1,000 m in central Hokkaido. The difference in population genetics between the two species would be supported by

Table 2. Inferences of historical processes

Clade	Sreps in inference key	Inferred event	χ^2	P-value
1-1	1-2-3-5-15-No	past fragmentation	21.00	0.0030
1-3	1-2-3-4-No	restricted gene flow	46.13	0.0200
2-1	1-2-3-4-No	restricted gene flow	14.60	0.0130
2-3	1-2-3-5-15-No	past fragmentation	12.00	0.0020
3-1	1-2-3-5-16-No	allopatric fragmentation	50.59	0.0000

χ^2 -test of geographical association of clades and inferences of biological causes for such association.

The inferences were obtained following the most updated key (Jan 06, 2011).

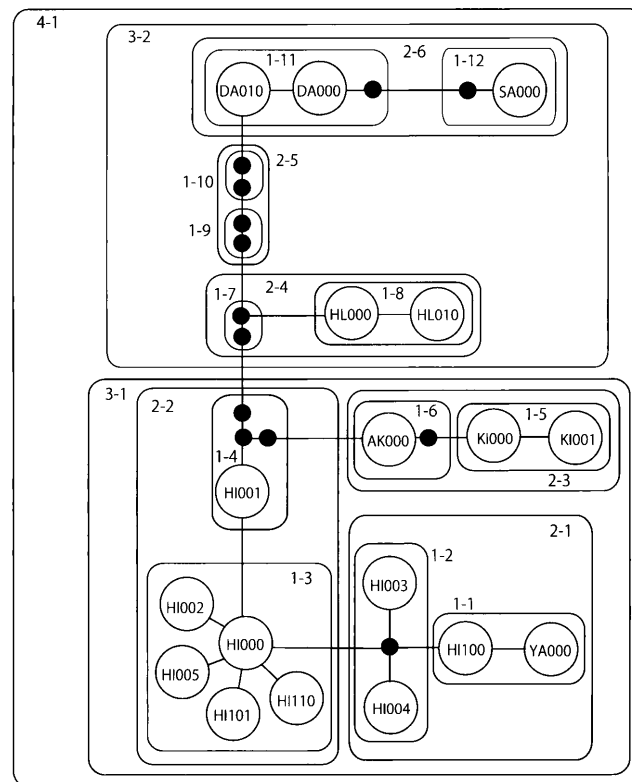


Fig. 5. Haplotype network and associated nested design.
All haplotypes are nested in the 4th level clade.

the difference in their ecological behavior described above.

As outlined above, *E. ligea* and *neriene* seem to have undergone different histories of distribution transitions in some localities in the Japanese archipelago, although they are closely related species of the same genus and prefer similar habitats. Similar examples of closely related species having a different era or location in which reproductive isolation took place, are also observed in Europe. *Erebia medusa* is found in grasslands in coniferous forests and in alpine meadows above the timberline in Bulgaria.

Schmitt *et al.* (2007) studied the populations from Romania and Bulgaria and pointed out that the species is separated into two distinct lineages, north and south, separated by the Danube valley. Despite the species having expanded its range after the last ice age, no genetic exchange occurred across the river valley. *Erebia euryale* is also found in grassland in the coniferous forest. The Romanian (the Carpathian mountains) and the Bulgarian (the Rila mountains) populations are, however, genetically very similar, suggesting that genetic exchanges across the Danube valley took place even after the last glacial period

1st Step Clade			2nd Step Clade			Total Cladogram		
No	Dc	Dn	No	Dc	Dn	No	Dc	Dn
1-1			2-1			Total		
YA000*	0.0	32.40S	1-1*	39.85S	40.71S			
HI100	3.78S	51.73L	1-2	0.0	65.20L			
I-T	3.77S	19.34L	I-T	-39.85	24.49L			
1-3			2-2			2-1*	70.01L	73.81L
HI002*	0.0	46.06	1-4	0.0	45.40	2-2	18.81S	59.78
HI005*	0.0	18.91S	1-3	41.61	41.38	2-3*	0.0S	44.36S
HI101*	0.0	28.17	I-T	not exist		I-T	-413.86	-584.65S
HI110*	0.0	28.17	2-3					
HI000	53.44L	53.42L	AK000	0.0S	20.94L			
I-T	53.55L	23.81L	KI000*	0.0S	20.92S			
			I-T	0.0	0.02L			

Fig. 6. Analytical results for NCPA with significant geographic structures.

Nesting hierarchy proceeds from the most recent 1st level clades on the left to the older clades on the right. Within each clade (boxed), columns indicate haplotypes, one hierarchical lower level clade or interior-tip contrasts, within clades distance (Dc), and nested clades distance (Dn). Individual haplotypes/clades with asterisks are tips. Significantly large and small statistical evaluations are denoted by L and S, respectively.

(Schmitt and Haubrich, 2008). *E. ligea* is also found in grassland in coniferous forest and is often sympatrically distributed with *E. euryale*, but a strong differentiation between the northern and southern mountain ranges in Bulgaria has been shown (Nakatani *et al.*, 2012).

As mentioned above, for different species even sharing a similar habitat in a particular region in the continent, different genetic population structures were found, which had probably formed in response to the various glacial cycles during the Quaternary period. In the Japanese archipelago, different genetic structures among different species may also be observed derived from species-specific biological abilities to adapt to climatic changes in the Quaternary period.

4.3 The ecological significance of the Harinoki Gap

As mentioned previously, a sympatric distribution area of the two sub-lineages of *E. ligea* was found in the Hida Mts., located at the Harinoki Gap (Fig. 4). *Oeneis norna* is a true alpine butterfly found only in the alpine zone above 2,700 m alt., the screes and dwarf pine (*Pinus pumila*) areas. In the vicinity of the Harinoki Gap, *O. norna* is divided into a northern sub-lineage distributed from Mt. Yukikuradake to Mt. Nunobikiyama and a southern sub-lineage distributed further south of Mt. Eboshidake, and is absent between Mt. Jigatake and Mt. Eboshidake

(Usami *et al.*, 2011). In addition, a unique lineage of *E. neriene* found in the Hida Mts. is spreading over the Harinoki Gap.

The distribution patterns of these three alpine butterflies around the Harinoki Gap can well be understood mainly as the reflection of species-specific fitness for certain altitudes (Fig. 8). Observing the geography and vegetation of the Harinoki Gap (Fig. 9), a large portion of the ridge is covered with coniferous and broad-leaved forests (*Betula ermanii*) due to the low altitudes of 2,500–2,600 m; the western side of the ridge is windward to the winter monsoon causing the forest to grow up to the ridge, with the leeward eastern side consisting of a steep and collapsed the topography scraped by avalanches (Fig. 9A), with narrow ridges with sheer sides sporadically present. On the very steep and collapsed slope, no grasses grow and even no transitional grasslands of *Poa* or *Carex* grass vegetation can be found. Only in the area around the summit of Mt. Rengedake there exists a topography of wide and gentle gravel areas with large colonies of *Dicentra peregrina* with very few *Pinus pumila* (Fig. 9F). These factors suggest that the distribution of alpine butterflies, *O. norna*, *E. ligea*, and *E. neriene* that feed on *Poa* and/or *Carex* grasses, is also restricted by the geography and vegetation around the Harinoki Gap. Another explanation of the vicarious distribution of *O. norna* in the Harinoki Gap has been

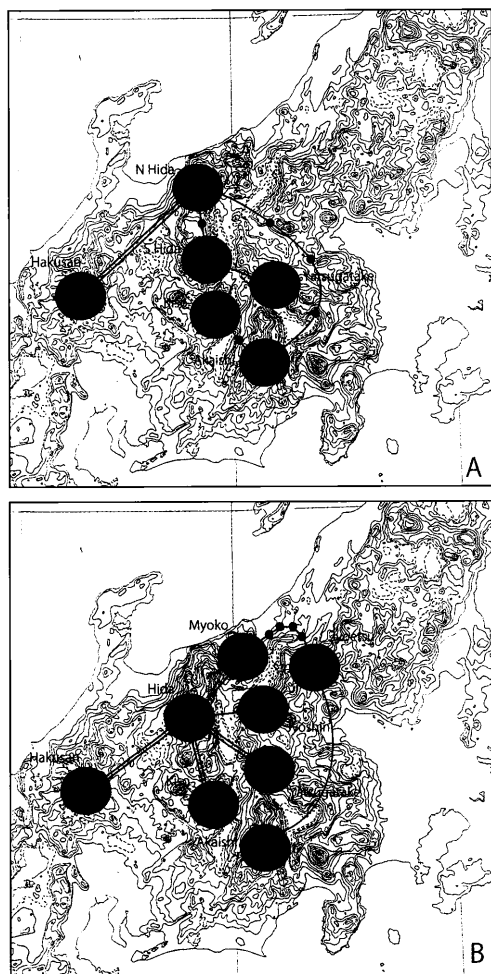


Fig. 7. Schematic distributions of major haplotypes and their phylogenetic relations in central Honshu. Circles denote haplotypes, small solid circles denote missing or theoretical haplotypes, a node represents a single mutational step, and a double line represents no mutational step. A: *Erebia ligea*, B: *Erebia neriene*. The distribution of *Erebia neriene* was drawn based on the data from Nakatani *et al.* (2007a).

proposed, in which it was caused by the sedimentation of volcanic products of Mt. Tateyama approximately 100,000 years ago (Itoh, 2010). However, the distribution patterns of these three alpine butterflies strongly suggest that species-specific ecological factors were the major cause.

The process of range expansion and contraction of alpine organisms due to climatic changes in the Quaternary period has been well known as a north-south migration in the Japanese archipelago (Ono, 1990). However, it could be possible that more than one refugium existed in the Japanese archipelago because of its complex mountain topography and the resulting micrometeorological environments. As a result of isolation by taking refuge in more than one refugium during the warm periods, genetically differentiated populations have survived till now as multiple lineages by expanding and contracting their distribution areas in adaptation to later glacial cycles (the multiple refugia model). The first example of such a multiple refugia model was suggested in the study on an alpine butterfly *Erebia neriene* (Nakatani *et al.*, 2007a). The present study also suggests the multiple refugia model for another alpine butterfly *E. ligea*, and it is further suggested that more organisms may well be subject to the same considerations.

Acknowledgments

Most of the distribution ranges of *Erebia ligea* in Japan are located within the Special Protected Zones of National Parks or Quasi-National Parks, and all animals and plants are protected. Also this species is designated as a Natural Monument by Nagano prefecture. Therefore permissions for collecting samples were granted by Ministry of the Environment, the Agency for Cultural Affairs, and the Nagano prefecture government. We were also provided with samples from Hokkaido by the National Institute for Basic Biology, and permissions were granted by related organizations. The samples from Nagano prefecture were

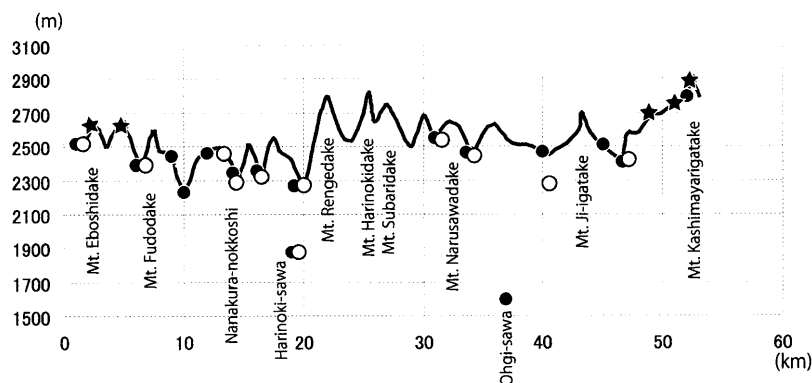


Fig. 8. Vertical distributions of three alpine butterflies in the Harinoki gap, in the central Hida Mts. Solid circle: *Erebia neriene*, open circle: *Erebia ligea*, star: *Oeneis norna*.

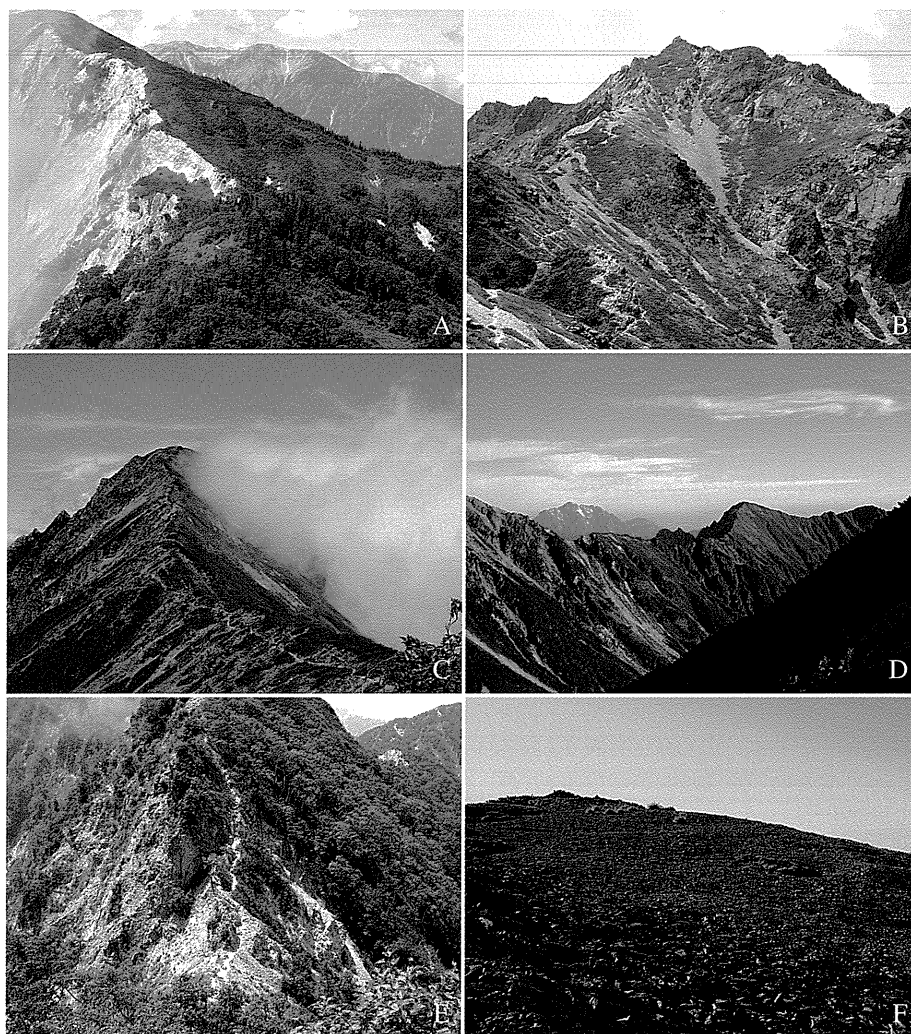


Fig. 9. Landscapes around the Harinoki gap, in the central Hida Mts.

- A: Steep ridge near Mt. Fudodake. East (left) side with collapsed cliff, west side covered with tall forest, with no grassland.
- B: Rocky cliff, between Mt. Subaridake and Mt. Harinokidake. Sporadic dried herbaceous communities, no *Erebia* species.
- C: Mt. Akazawadake overlooked from Mt. Subaridake. Some alpine grassland spreads on the leeward slope (right, east side). *Erebia* species and *Colias palaeno* inhabit.
- D: Mt. Subaridake (left) to Mt. Akazawadake and Mt. Narusawadake (right, NNE). Light green zones near the ridge are grasslands where *Erebia* species are found.
- E: Saddle point area, Nanakura-nokkoshi (right, east side). *Erebia* species found.
- F: Wide and gentle ridge near the summit of Mt. Rengedake. *Dicentra peregrina* (pink flowers) abundant and some *Carex*. No alpine butterflies.

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摘 要

第四紀日本列島におけるクモマベニヒカゲの分布変遷史 (中谷貴壽・宇佐美真一・伊藤建夫)

日本列島は氷河期には大陸と繋がっていた時代があり、その期間には大陸起源の北方系生物は分布を拡大し、間氷期にはこれらの生物は大陸へ避難し、あるいは一部の個体群を除いて絶滅したと考えられてきた。しかし、筆者らの高山蝶に関する研究によると、ベニヒカゲは古い時代の氷河期に渡来し、間氷期にも日本列島の高山帯で複数の個体群が生き残り、互いに生殖隔離された結果列島内で複数の系統に分化した後に、次の氷河期に分布を拡大するというサイクルを繰り返してきたことが明らかになった(複数レフュジア・モデル)。これはツツバシオガマ他数種の高山植物で明らかにされたシナリオ、初めの氷河期に大陸から渡来した系統が間氷期に本州中部山岳で生き残り、次の氷河期に新たに侵入した系統が東北地方以北に分布するという

時間差侵入仮説（単一レフュジア・モデル）とは異なる。

本研究ではサハリンを含む日本列島から17のハプロタイプを見出した（Table 1）。複数地域または複数サンプルから検出された系統的意義を有するハプロタイプは、本州では飛騨山脈北部・白山、飛騨山脈南部、八ヶ岳、木曽山脈、赤石山脈の5系統に、また北海道では大雪山の高標高部と山麓の低標高部の2系統に分かれている（利尻島高山帯にも孤立した個体群が分布するが未検、Fig. 3）。サハリン、北海道、本州の個体群は、過去の異所的分断により分断分布を成している事が明らかである。本州では飛騨山脈で複数のハプロタイプが混生しており（Fig. 4）、複数のイベントによって現在の分布が形成されたと考えられるので、NCPAにより過去の分布変遷史を推定した結果、5つのクレードで統計的に有意なイベントが推定された（Table 2）。それによると、クモマベニヒカゲの中部山岳地域における分布の変遷は、分断と拡散の繰り返しであることが示唆された。

日本列島における分布変遷

日本列島へ進出したクモマベニヒカゲの個体群は、サハリン・北海道・本州の現在の分布域を包含する地域に分布を広げた。その後の温暖期にサハリン、北海道、本州のレフュジアに分断され、それぞれが別々の系統に分化した。次の温暖期に、本州の中部山岳地域では飛騨山脈北部系統、同南部系統、赤石山脈・木曽山脈系統の3系統に分断された。続く氷河期に分布を拡大し、飛騨山脈の北部系統と南部系統は、後立山連峰の針ノ木岳付近（以後針ノ木ギャップと呼ぶ）で混生地帯を形成した。さらにその後の温暖期に現在見るような離散分布が形成された。

ベニヒカゲとの比較による系統地理的な特徴

広域に分布するハプロタイプの系統関係を概観すると（Fig. 7）、両種ともに飛騨山脈と赤石山脈産のハプロタイプ間に大きな遺伝的差異のあることが示される。初期の単一な遺伝的組成をもつ集団が、その後の温暖期に分布を縮小する過程で飛騨山脈と赤石山脈に分布する二つの個体群に分断された結果、二つの系統に分岐したことを示唆している。飛騨山脈と赤石山脈のレフュジアに源を発する2系統の遺伝的距離は、ベニヒカゲとクモマベニヒカゲとの間で差異があり、分岐年代には若干の差があったと考えられる。またベニヒカゲとクモマベニヒカゲ共に、赤石山脈の系統は、飛騨山脈系統の北部集団とより近縁である事は、その後の気候変動に適応して分布を拡大したルートが両種で類似している可能性を示唆している。

飛騨山脈では、ベニヒカゲは単一のハプロタイプが産するが、クモマベニヒカゲでは針ノ木ギャップで混生地帯を挟んで南北2系統に分かれており、両系統の間に2塩基の差が認められる点が大きく異なる。白山山系は飛騨山脈とは地理的に非常に離れているがベニヒカゲ、クモマベニヒカゲ（クモマベニは飛騨山脈の北部系統）共に同じハプロタイプが分布しており、二つの山系の個体群間ではきわめて最近まで遺伝的交流があったことが示唆される。八ヶ岳では、ベニヒカゲは飛騨山脈と同一のハプロタイプが、またクモ

マベニヒカゲは飛騨山脈の南部系統と1塩基差の近縁なハプロタイプが見出され、両地域の個体群の近縁性が示唆される。これに対して木曽山脈では、ベニヒカゲは飛騨山脈と共通のハプロタイプが見出されるのに対して、クモマベニヒカゲでは赤石山脈と近縁であり、木曽山脈の個体群の分布変遷は両種の間で異なっていた可能性が示唆される。一方サハリンと北海道の個体群に関しては、ベニヒカゲは共通のハプロタイプが分布するなど、最近まで遺伝的交流があったことが示唆されるが、クモマベニヒカゲの個体群は遺伝的に非常に異なっており、古くから生殖隔離が続いていることが示唆された。

ベニヒカゲとクモマベニヒカゲは同じ属に含まれる近縁種であり、また生息環境も似ているが、いくつかの地域では異なる分布変遷史をたどったようだ。このように種によって生殖隔離の始まった時期や場所が異なる事例はヨーロッパでも知られている。*Erebia medusa*はヨーロッパでは針葉樹林内の草原を主たる生息地としているが、ルーマニアとブルガリアの28集団について調べた研究によると、ドナウ河を挟んで南北2系統に分断されており、最終氷期にそれぞれの集団が分布を拡大したもののドナウ河を越えて遺伝的交流が成されることはなかったとしている。一方*E. medusa*と同様に針葉樹林内の草原を主たる生息地として広域分布する*Erebia euryale*では、ルーマニア産とブルガリア産は遺伝的によく似ており、最終氷期以降にドナウ河を越えて遺伝的交流があったとする研究がある。このように大陸内でしかも生息環境が類似した種でも、第四紀の氷河サイクルに対する適応は種によって異なる事例があるように、日本列島内における第四紀の気候変動に対する高山蝶の適応は、種特異的ないろいろなパターンが存在することが強く示唆される。

針ノ木ギャップの生態的意義

すでに述べたように、飛騨山脈におけるクモマベニヒカゲのハプロタイプは、鹿島槍ヶ岳から烏帽子岳に至る針ノ木ギャップで2系統の混生地帯が見られる（Fig. 4）。タカネヒカゲは標高約2,700 m以上の岩礫帯からハイマツ帯にかけての高山帯にのみ生息する真性高山蝶で、針ノ木ギャップ付近では爺ヶ岳から烏帽子岳の間で分布を欠いており、雪倉岳から鹿島槍ヶ岳・布引山にかけて分布する北部系統と、烏帽子岳以南に分布する南部系統に分岐している。一方、ベニヒカゲは針ノ木ギャップを含む飛騨山脈全域に単一の系統が分布する。針ノ木ギャップ付近におけるこれら3種の高山蝶の分布状況は、種の標高に対する適応の度合いを反映したものとなっている（Fig. 8）。針ノ木ギャップ付近の地形および植生をみると（Fig. 9）、全体に標高が約2500–2600 mと低いために尾根の多くが樹林帯で覆われており、冬季季節風の風上に当たる尾根の西側では樹林が尾根まで迫り、風下側の東側は雪崩によって削られた急峻な崩落地形となり、あるいは両側の切り立った狭い尾根が断続的に見られる。急峻な崩落地にはイネ科やカヤツリグサ科の遷移途上の草地すら見られない。蓮華岳の頂上付近にのみ広い緩傾斜の砂礫帯があり、コマクサが大群落を形成

するがハイマツはあまり生えていない。このような植生が、イネ科やカヤツリグサ科植物を食草とする高山蝶たち（タカネヒカゲ、クモマベニヒカゲ、ベニヒカゲ）の分布を規制しているものと考えられる。針ノ木ギャップ付近におけるタカネヒカゲの不連続分布の原因を約10万年前の立山噴火による噴出物の堆積に求める説もあるが、3種の高山蝶にみられる分布パターンは、それぞれの種が持つ高度適応力の強さを反映しており、生態的要因がより強く働いた結果であると考えられる。

従来は日本列島の生物相形成に関して、大陸からの複数回

の進出によって氷河サイクルに適応したとする事例（単一レフュジア・モデル）が指摘されるケースが多かった。しかし筆者らによるベニヒカゲやタカネヒカゲ、さらには今回報告したクモマベニヒカゲの研究で示唆されるように、高山性生物の種によっては古くから日本列島に侵出し、日本列島内で氷河サイクルを通じて分布の分断・拡張を繰返してきたケース（複数レフュジア・モデル）が少なくないことが伺われる。

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